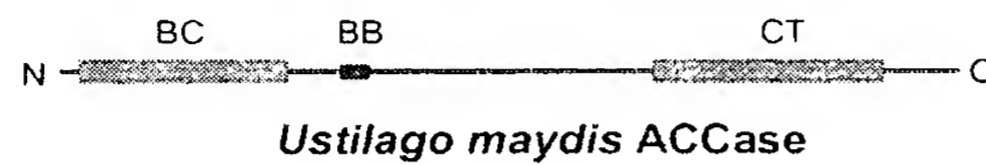


Figure 1

ACCase: Large, Complex & Labile



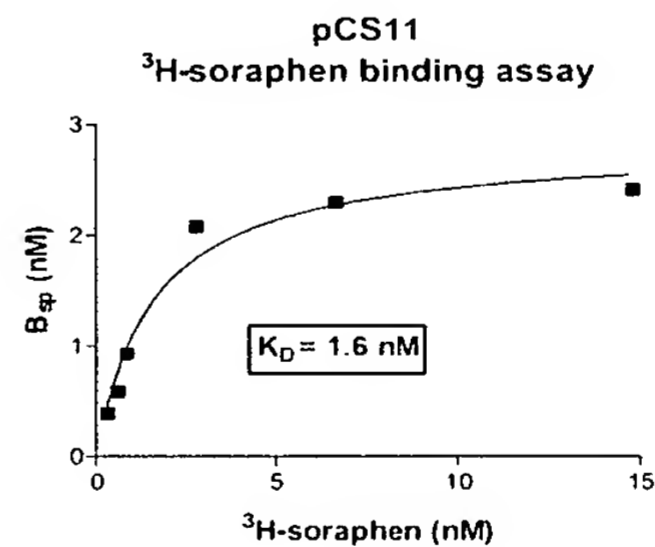
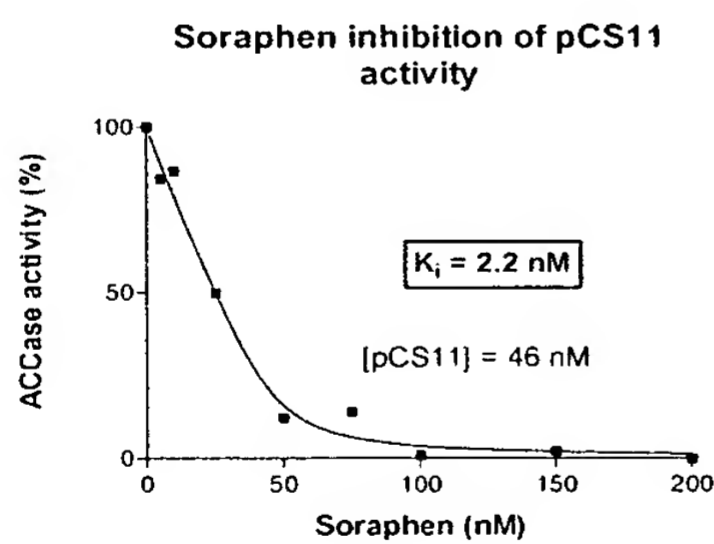
- 3 functional domains; 2 enzymatic reactions:
 - BB=biotin binding
 - BC=biotin carboxylase (*site of soraphen inhibition)
 - CT=carboxy transferase

net reaction: acetyl CoA + CO₂ → malonyl CoA

- low abundant and labile protein

Figure 2

Soraphen Binds to and Inhibits recombinant full-length *Ustilago* ACCase (pCS11 protein)



Soraphen inhibition of endogenous *U. maydis* ACCase: $K_i = 1.4 \text{ nM}$
(Heike Behrbohm Ph.D. thesis, Braunschweig Techn. Univ., 1996)

Figure 3

pCS8 Binds Soraphen with Similar Affinity as pCS11

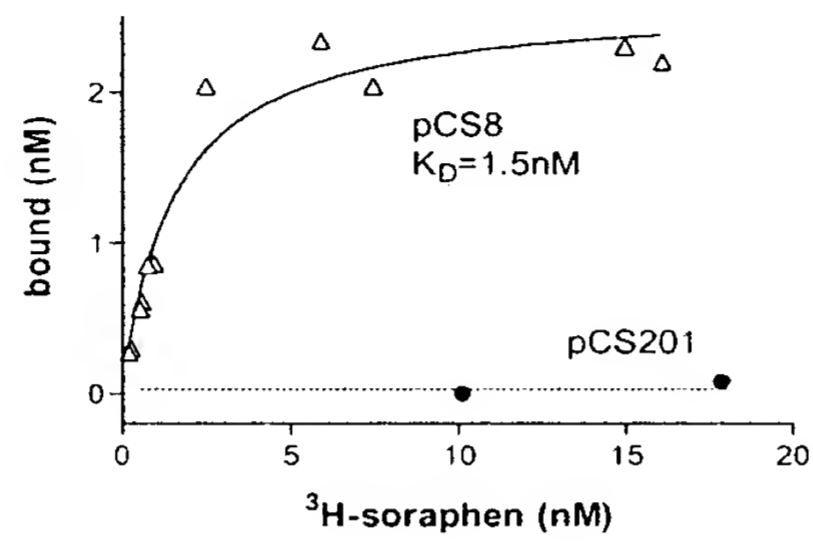


Figure 4

**Soraphen Binding by a Soluble
Phytophthora infestans BC domain**

- Expressed as N-terminal His-tagged protein
- 54% identical, 67% similar to *Ustilago* BC domain
- Exhibits high-affinity soraphen binding
- Use as additional partition agent to select for broad specificity

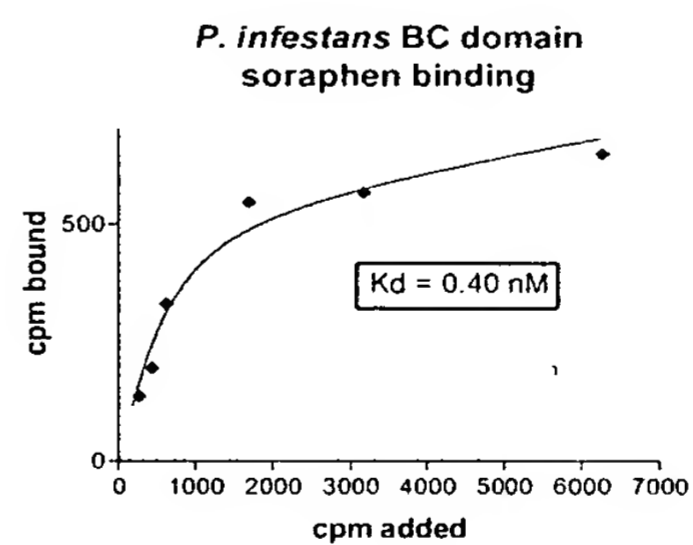
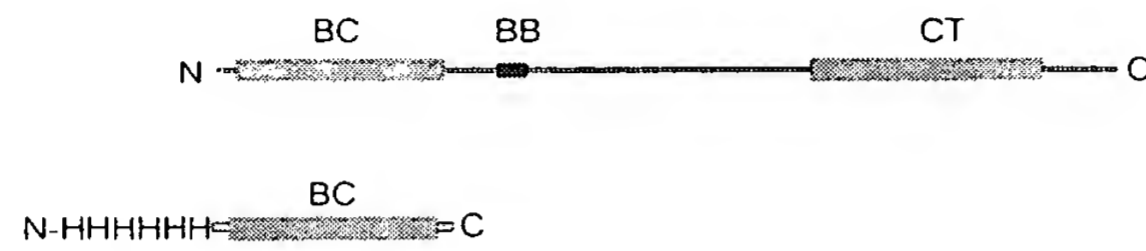


Figure 5

pCS8: Small, Simple & Stable



- N-terminal His-tag to facilitate purification
- high expression in E.coli: 20-50 mg per 1 liter culture
- very stable under laboratory conditions

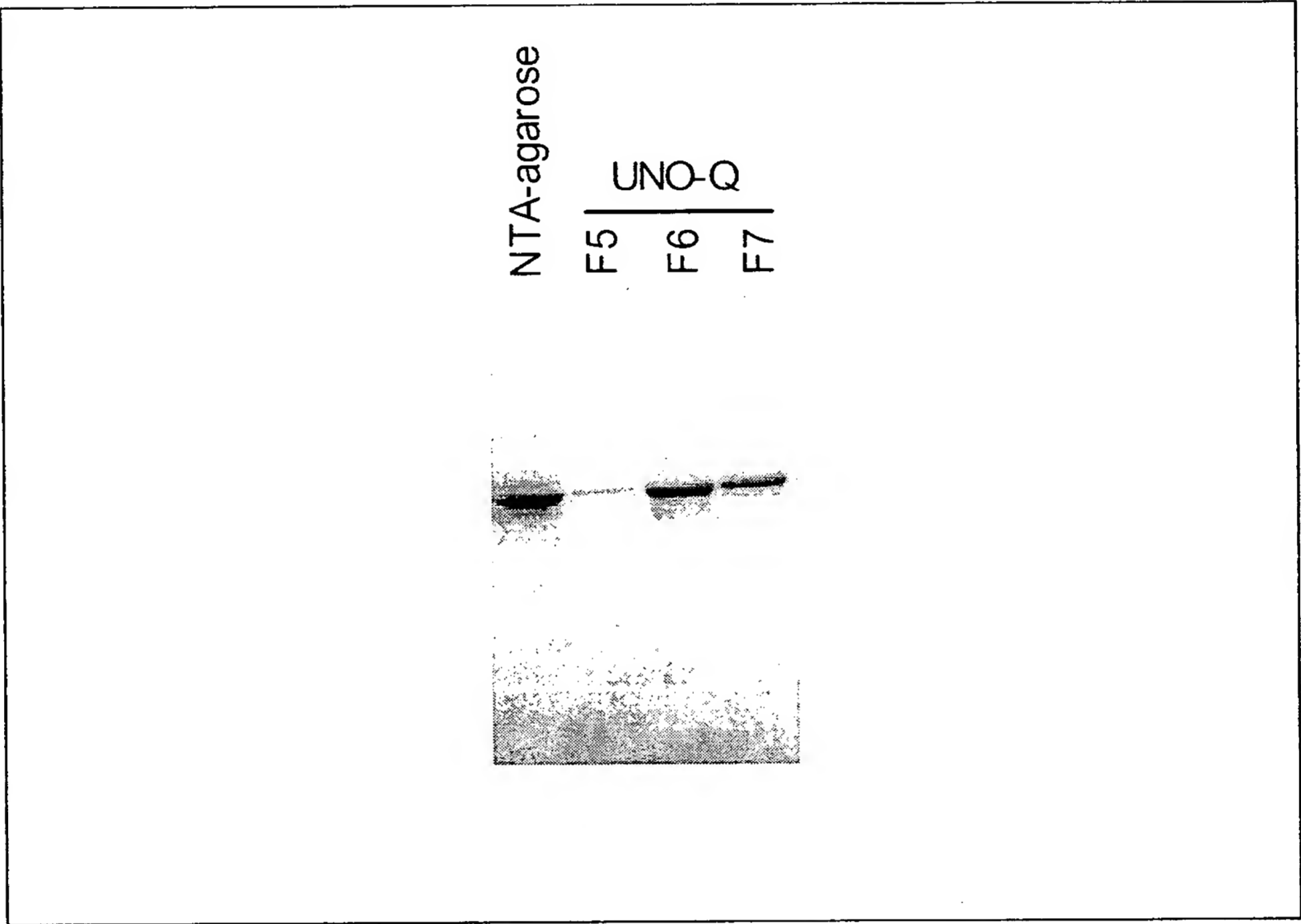
PPPDHKAVSQFIGGNPLETAPASPVADFI RKQGGH SVITKVLI
CNNGIAAVKEIRSIRKWAYETFGDERAIEFTVMATPEDLKVNA
DYIRMADQYVEVPGGSNNNNYANVDLIVDVAERAGVHAVWAGW
GHASENPRLPESLAASKHKIIFIGPPGSAMRSLGDKISSSTIVA
QHADVPCMPWSGTGIKETMMSDQGFLT VSDDVYQQACIHTAEE
GLEKAEKIGYPVMIKASEGGGGKGIRKCTNGEEFKQLYNAV LG
EVPGSPVFVMKLAGQARHLEVQLLADQYGNAISIFGRDCSVQR
RHQKII EEAPVTIAPEDARESM EKAAVRLAKLVGYVSAGTVEW
LYSPESGEFAFLELNPRLQVEHPTTEMVSGVNI PAAQLQVAMG
IPLYSIRDIRTLYGMDPRGNEVIDFDFSSPESFKTQRKPQPQG
HVVACRITAENPDTGFKPGMGALTELNFRSSTSTWGYFSVAAS
GALHEYADSQFGHIFAYGADRSEARKQMVISLKELSIRGDFRT
TVEYLIKLLETDAFESNKIT TGWLDGLIQDRLTAERPPADLAV

**Figure 6. Amino acid sequence of *Ustilago maydis* ACCase
BC Domain (Amino Acids 2-560) (also SEQ ID NO:2)**

MVAEEAPPAADVAAYAETRSDSNPLNYASMEEYVRLQKGTRPITSVL
IANNGISAVKAIRSIRSWSYEMFADEHVVTFFVVMATPEDLKANA EYI
RMAEHVVEVPGGSNNHNYANVSLII EIAERFNVD AVWAGWGHASENP
LLPDTLAQTERKIVFIGPPGKPMRALGDKIGSTIIAQSAKVPTIAWN
GDGMEVDYKEHDGIPDEIYNAAML RDGQHCLDECKRIGFPVMIKASE
GGGGKGIRMVHEESQVLSAWEAVRGEIPGSPIFVMKLAPKSRHLEVQ
LLADTYGNAIALSGRDCSVQRRHQKIVEEGPVLAPTQEVWEKMMRAA
TRLAQEVEYVNAGTVEYLFSELPEDNGNSFFFLELNPRLQVEHPVTE
MITHVNLPA AQLQVAMGIPLHCI PDVRRLYNKDAFETTVIDFDAEKQ
KPPHGHVIAARITAEDPNAGFQPTSGAIQELNFRSTPDVWGYFSVDS
SGQVHEFADSQIGHLFSWSP TREKARKNMVLALKELSIRGDIHTTVE
YIVNMME SDDFKYNRISTSWLDERISHHNEVRLQGRPD

**Figure 7: Amino acid sequence of *Phytophthora infestans*
ACCcase BC Domain (Amino Acids 1-555) (also SEQ ID NO:4)**

Figure 8: Anion Exchange Chromatography of pCS8 showing the Ni-NTA-agarose input and the peak fractions (F) off of the UNO-Q column (anion exchange column).



Spectrophotometric assay of *E. coli* BC

Assay absorbance traces

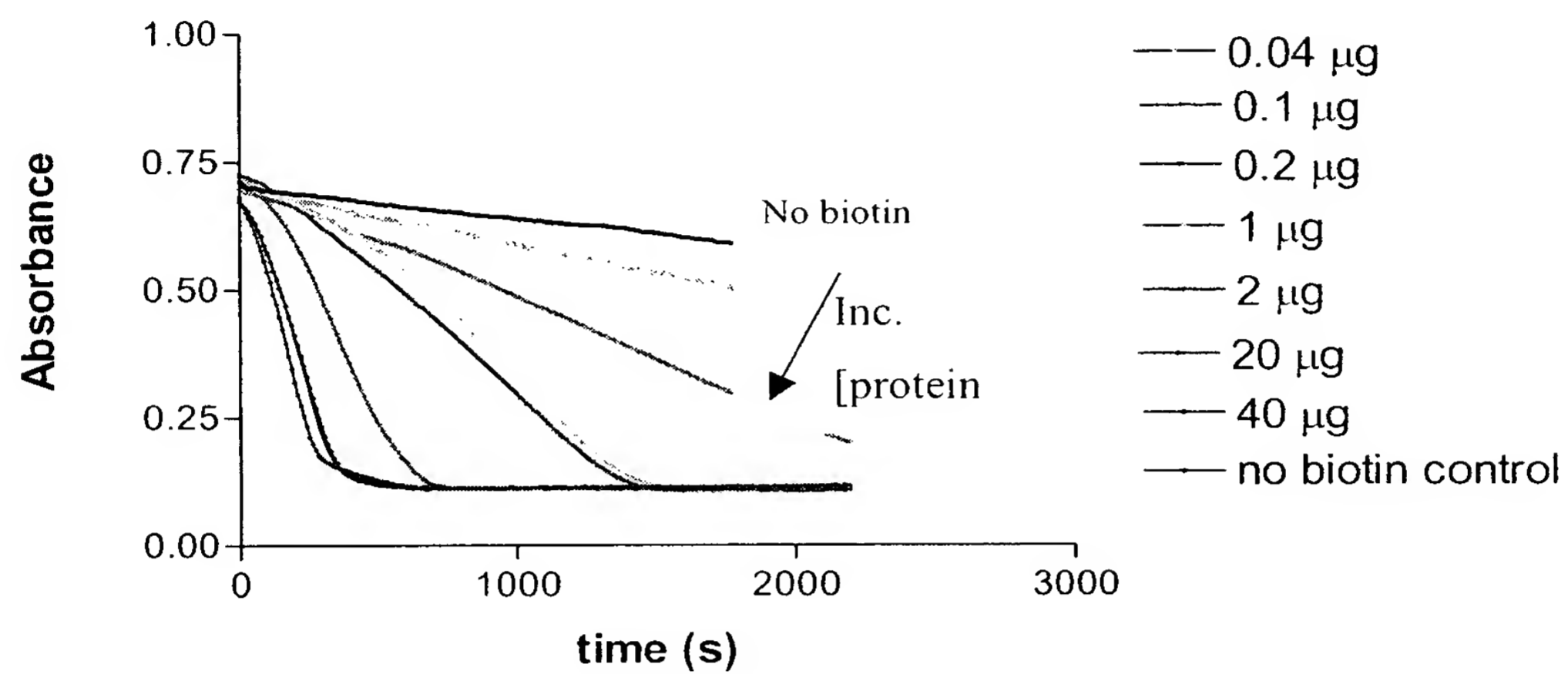


Figure 9A

Activity Assays using full-length recombinant Ustilago

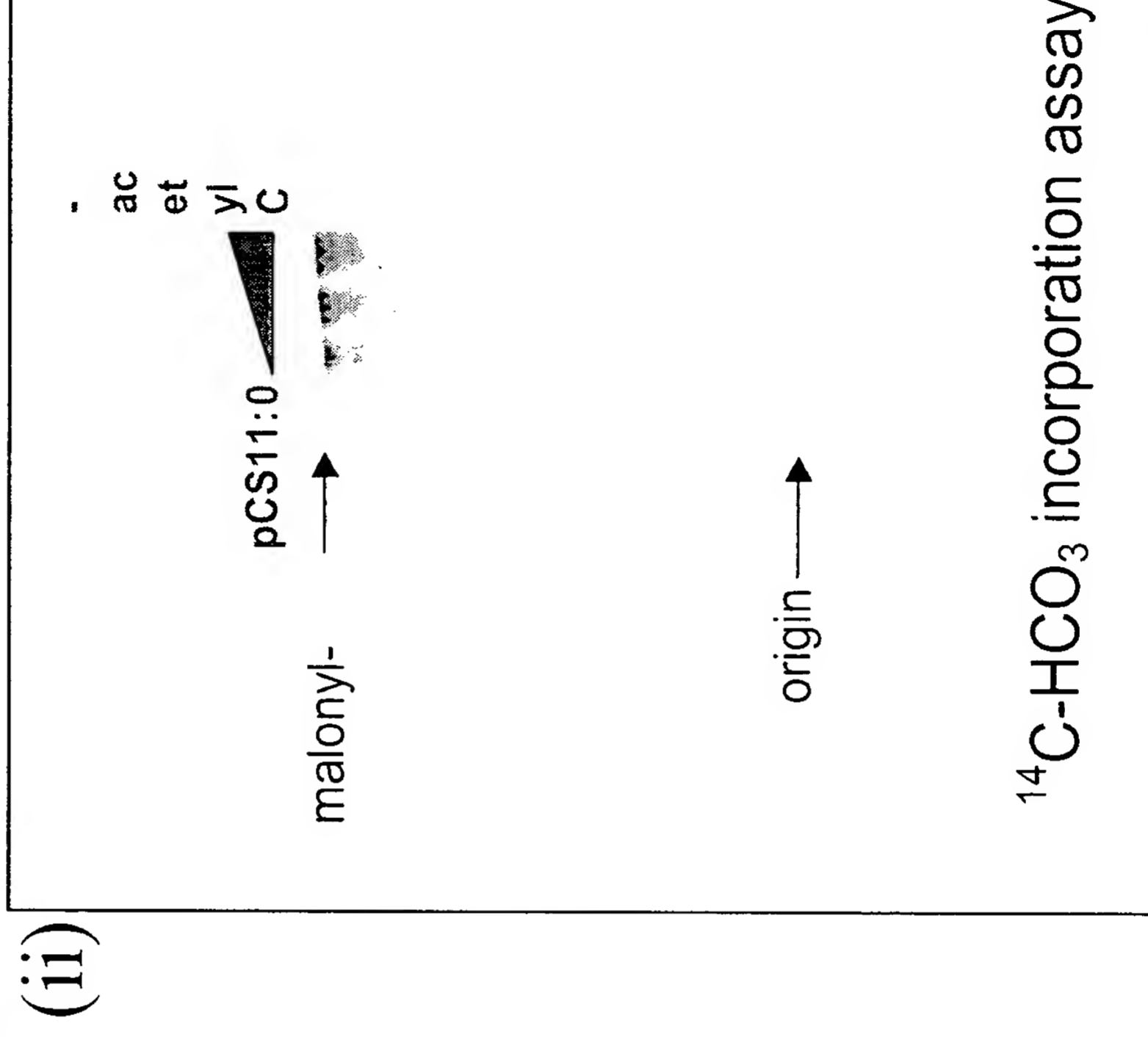
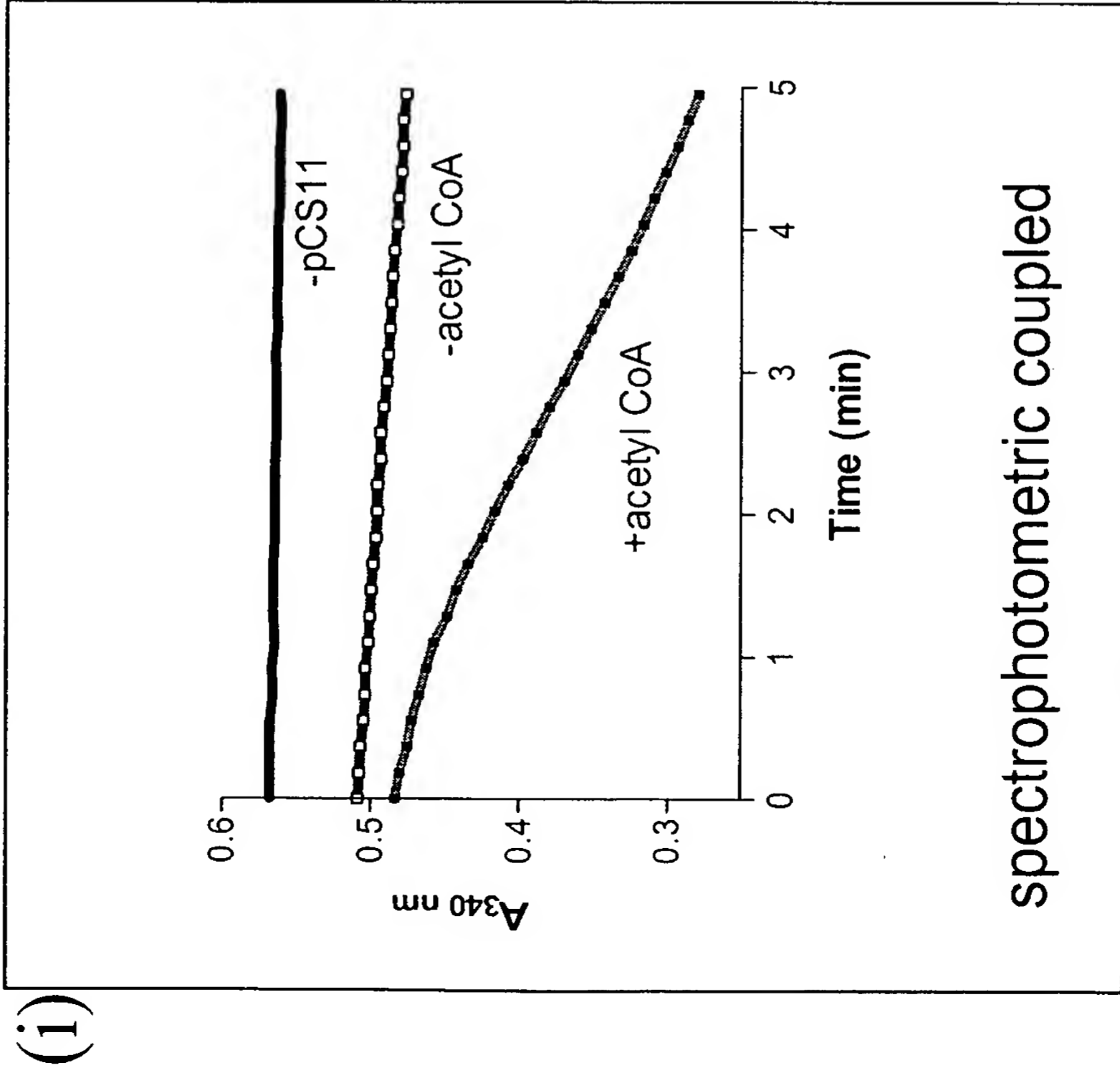
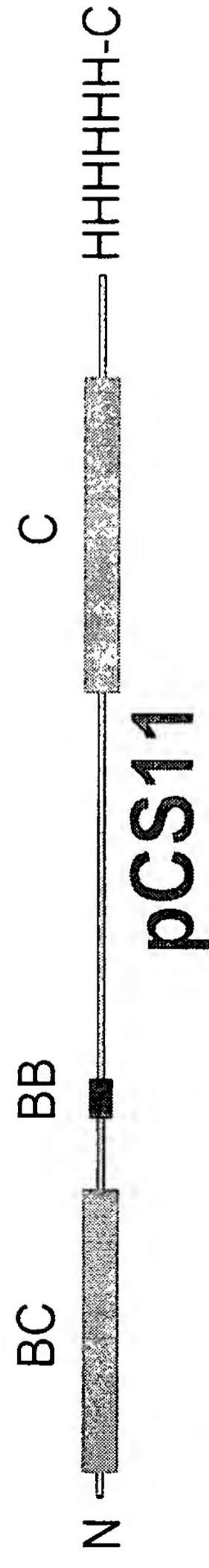


Figure 9B

ustilago	-----PPPD-----HKAVSQ-----	FIG-GNP
phytophthora	-VAEEAP-----PAAD-----	TRSDSNP
yeast	SEESLFESS-----PQKM-----	EYEITNYSERHTELPGHFIG-LNT
magnaporthe	TETNGTAAANSSRQRNGANGVTPVANGKATYAQRHKIADH-----	FIG-GNR
		* *

ustilago
phytophthora
yeast
magnaporthe

FIG. 10 (part 1 of 4)

ustilago	RLPESLAASKHKIIFIGPPGSAMRSLGDKISSSTIVAQHADVPCMPWSGTGIKETMMSDQ-
phytophthora	LLPDTLAQTERKIVFIGPPGKPMRALGDKIGSTIIAQSAKVPTIAWNGDGMEDVDYKEHD-
yeast	LLPEKLSQSKRKVIFIGPPGNAMRSLGDKISSSTIVAQSAKVPCIPWSGTGV-DTVHVDEK
magnaporthe	KLPESLAASPKKIIFIGPPGSAMRSLGDKISSSTIVAQHAQVPCIPWSGTGVDAVQIDKK-
	** * * * * ** ** ** **
ustilago	-GFLTVSDDVYQQACIHTAEEGLEKAEKIGYPVMIKASEGGGKGIRKCTNGEEFKQLYN
phytophthora	-G-- -IPDEIYNAAMLRDQGHCLDECKRIGFPVMIKASEGGGKGIRMVHEESQVLSAWE
yeast	TGLVSVDDDIYQKGCCTSPEDGLQAKKRIGFPVMIKASEGGGKGIRQVEREEDFIALYH
magnaporthe	-GIVTVDDDTYAKGCVTSWQEGLEKARQIGFPVMIKASEGGGKGIRKAVSEEGFEEELYK
	* * * * *
ustilago	AVLGEVPGSPVFMKLAGQARHLEVQLLADQYGN AISIFGRDCSVQRRHQKIEEAPVTI
phytophthora	AVRGEIPGSPIFVMKLAPKSRHLEVQLLADTYGNAIALSGRDCSVQRRHQKIVEEGPVL A
yeast	QAANEIPGSPIFIMKLAGRARHLEVQLLADQYGTNISLFGRDCSVQRRHQKIEEAPVTI
magnaporthe	AAASEIPGSPIFIMKLAGNARHLEVQLLADQYGNNISLFGRDCSVQRRHQKIEEAPVTI
	* * * * * ** ** * * * * *

FIG. 10 (part 2 of 4)

ustilago	APEDAREMEKAAVRLAKLVGYVSAGTVEWLYS--PESG--EFAFLELNPRLQVEHPTTE
phytophthora	PTQEVWEKMMRAATRLAQEVEYVNAGTVEYLFSELPEDNGNSFFLELNPRLQVEHPVTE
yeast	AKAETFHEMEKAAVRLGKLVGYVSAGTVEYLYS--HDDG--KFYFLELNPRLQVEHPTTE
magnaporthe	AKPDTFKAMEEAAVRLGRLVGYVSAGTVEYLYS--HADD--KFYFLELNPRLQVEHPTTE
	* * * * * * * * * * * * * * * * * *
ustilago	MVSGVNI PAAQLQVAMGIPLYSI RDIRTLYGMDPRGNEVIDFD FSSPESFKTQRKPQPG
phytophthora	MITHVNL PAAQLQVAMGIPLHCI PDVRRLLYNKDAFETTVIDFD-----AEKQKPPHG
yeast	MVSGVNL PAAQLQIAMGIPMHRI SDIRTLYGMPNPHSASEIDFEFKTQDATKKQRRPIPKG
magnaporthe	GVSGVNL PASQLQIAMGIPLHRI SDIRLLYGVDPKLSTEIDFD FKNPDSEKTQRRPSPKG
	** ** ** ** ** ** * * * * * * * * * *
ustilago	HVVACRI TAENPDTGFKPGMGALTEL NFRSSTSTWGYFSVGTSGALHEYADSQFGHIFAY
phytophthora	HVIAARI TAEDPNAGFQPTSGAIQELNFRSTPDVWGYFSVDSSGQVHEFADSQIGHLFSW
yeast	HCTACRI TSEDPNDFGFKPSGGTLHEL NFRSSSNVWGYFSVGNNGNIHSFSDSQFGHIFAF
magnaporthe	HLTACRI TSEDPGEGFKPSNGVMHEL NFRSSSNVWGYFSVGTQGGIHSFSDSQFGHIFAY
	* * * * * * * * * * * * * * * * * *

FIG. 10 (part 3 of 4)

ustilago	GADRSEARKQMVISLKELSIRGDFRRTTVEYLIKLLLETDAFESNKITTTGWLDGLIQDRLTA
phytophthora	SPTREKARKNMVLALKELSIRGDIHTTVEYIVNMMESSDDFKYNRISTSWLDERISHHNEV
yeast	GENRQASRKHMVVALKELSIRGDFRRTTVEYLIKLLLETEDEFEDNTITTTGWLDDLIITHKMTA
magnaporthe	GENRSASRKHMVIALKELSIRGDFRRTTVEYLIKLLLETEAFEENTITTTGWLDELISKKLTA

*	**	**	*****	*****	*	*	*	*	*	*	*	*	*	*
---	----	----	-------	-------	---	---	---	---	---	---	---	---	---	---

ustilago	E---	RPPADLAV	(SEQ	ID	NO:	2)
phytophthora	RLQGRPD	----	(SEQ	ID	NO:	4)
yeast	E---	KPDPTLAV	(SEQ	ID	NO:	8)
magnaporthe	E---	RPDKMLAV	(SEQ	ID	NO:	6)

*

FIG. 10 (part 4 of 4)

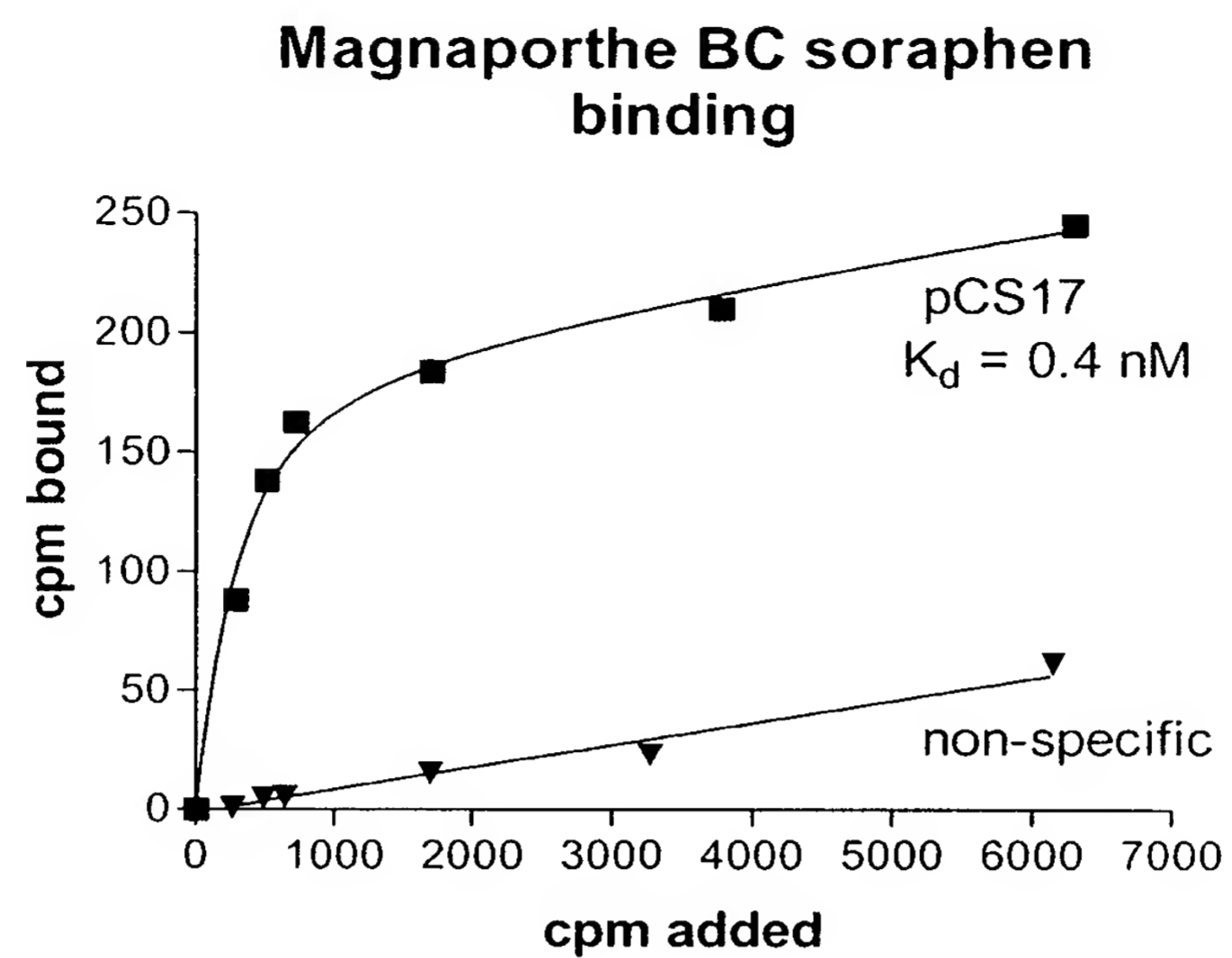


FIG. 11

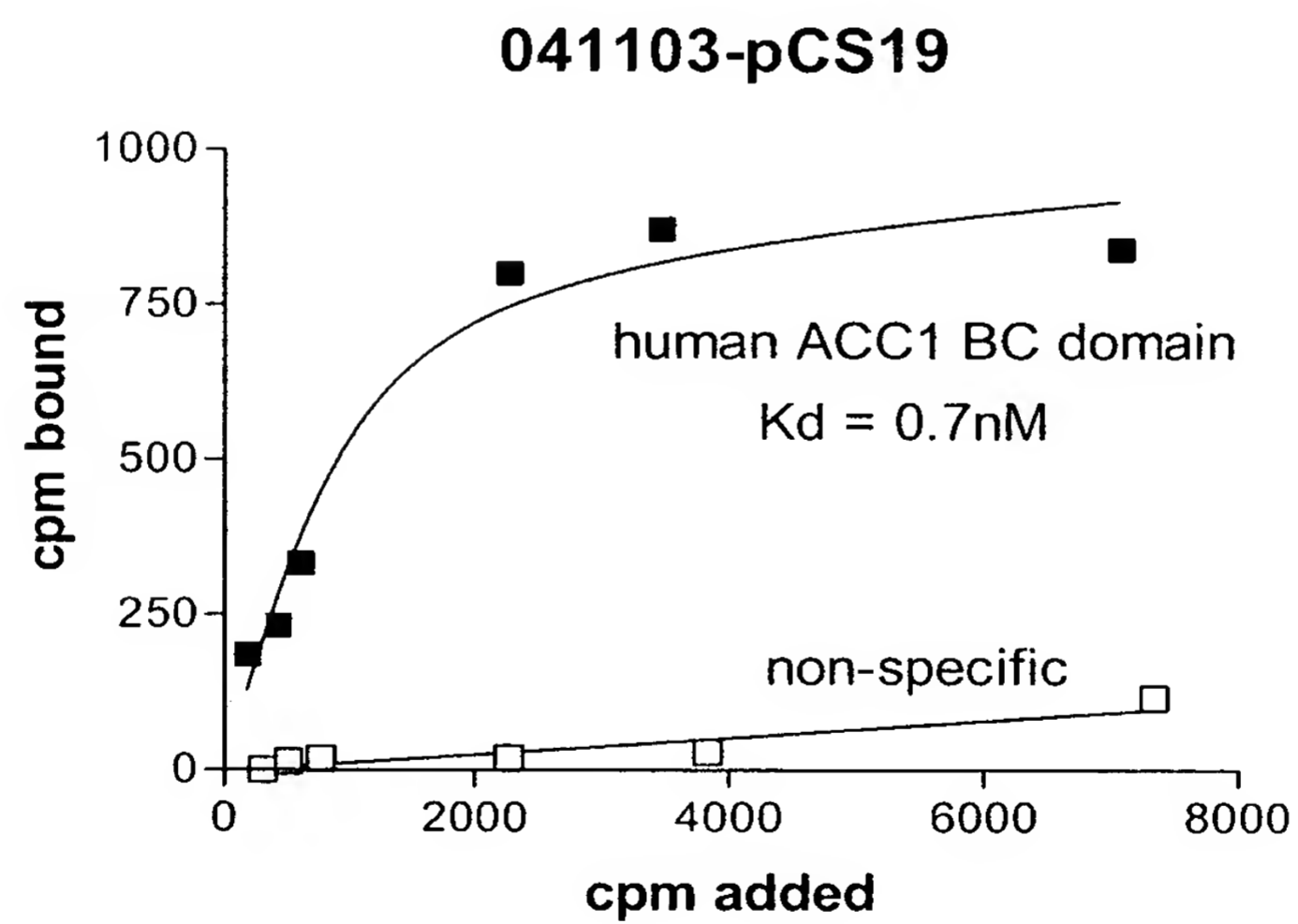


FIG. 12

Alignment of the ustilago and human ACCase BC domains

ustilagoBC	-----
ACC1BC	MDE-----
ACC2BC	MVLLCLSLIFSLTFSWLKIWGKMTDSKPITKSKSEANLIPSQEPFPASDNSGETPQR
ustilagoBC	-----PPPDHKAV-----S-----QFIGGNPLET-----
ACC1BC	-----PSPLAQPLELNQHS-----RFIGSVSEDNSEDEISNL
ACC2BC	NGEGHTLPKTPSQAEPA SHKGP-----KDAGRRRNSLP PSHQKPPRNPLSS-----
ustilagoBC	-----APAS-----
ACC1BC	VKLDLLEKEGSLSPASVGSDTLSDLGISSLQDGLALHIRSSMSGLHLVKQGRDRKKIDS
ACC2BC	-----SDAA-----
	*

FIG. 13 (part 1 of 5)

ustilagoBC	-----PV-----
ACC1BC	QRDFTVASP-----
ACC2BC	-----PSPELQANGTGTOGLEATDTNGLSSSARPQGGQAGSPSKEDKKQANIKRQLMT
ustilagoBC	-----
ACC1BC	-----
ACC2BC	NFILGSFDDYSSDEDSVAGSSRESTRKGSRASLGALSLEAYLTTGEAETRVPTMRPSMSG
ustilagoBC	-----
ACC1BC	-----ADFIKQGGHSVITKVLI CNNGIAAVKEIRSIRKWA
ACC2BC	-----AEFVTRFGGNKVIEKVLIANNGIAAVKCMRSIRRRWS
	LHLVKRGREHKKLDLHRDFTVASPAEFVTRFGGDRVIEKVLIANNGIAAVKCMRSIRRRWA
	* * * * *

FIG. 13 (part 2 of 5)

ustilagoBC	YETFGDERAIEFTVMATPEDLKVNADYIRMAHQYVEVPGGSNNNNYANVDLIVDVAERAG
ACC1BC	YEMFRNERAIRFVVMVTPEDLKANAHEYIKMADHYVPVPGGPNNNNYANVELILDI AKRIP
ACC2BC	YEMFRNERAIRFVVMVTPEDLKANAHEYIKMADHYVPVPGGPNNNNYANVELI VDI AKRIP
	** *
ustilagoBC	VHAVWAGWGHA SENPRLPESLAASKHKIIFIGPPGSAMRSLGDKISS TIVAQHADVPCMP
ACC1BC	VQAVWAGWGHA SENPKLPELLL--KNGIAFMGPPSQAMWALGDKIASSIVAQTAGIPTLP
ACC2BC	LQAVWAGWGHA SENPKLPELLC--KNGVAF LGPPSEAMWALGDKIASTVVAQTLQVPPTLP
	***** * * * * * * * * * * * * * * *
ustilagoBC	WSGTGIKETMMSD---QGF-LTVSDDVYQQACIHTAE EGLEKAEKIGYPVMIKASEGGGG
ACC1BC	WSGSGLRVDWQENDFSKRI-LNVPQELYEKGYVKD VDDGLKAAEEVGYPVMIKASEGGGG
ACC2BC	RS GSGLTVEWTEDDLQQGKRISVPEDVYDKGCVKD VDEGLEAAERIGFPLMIKASEGGGG
	** *

FIG. 13 (part 3 of 5)

ustilagoBC	KGIRKCTNGEEFKQLYNAVLGEVPGSPVFVMKLAGQARHLEVQLLADQYGNAISIFGRDC
ACC1BC	KGIRKVNADDDFPNLFQRQVAEVPGPSPIFVMRLAKQSRHLEVQILADQYGNAISLFGRDC
ACC2BC	KGIRKAESAEDFPILFRQVQSEIPGPSPIFLMKLAQHARHLEVQILADQYGNAVSLFGRDC
	***** * * * * ***** * * * * *
ustilagoBC	SVQRRHQKIIIEAPVTIAPEDARESMEKAAVRLAKLVGYVSAGTVEWLYPESGEFAFLE
ACC1BC	SVQRRHQKIIIEAPATIATPAVFEHMEQCAVKLAKMVGYSAGTVEYLYS-QDGSFYFLE
ACC2BC	SIQRRHQKIVEEAPATIAPLAIFEFMEQCAIRLAKTVGYVSAGTVEYLYS-QDGSFHFLE
	* ***** * * * * * ***** * * * *
ustilagoBC	LNPRLQVEHPTTEMVSGVNI PAAQLQVAMGIPLYSIRDITLYGMDPRGNEVIDFDFFSSP
ACC1BC	LNPRLQVEHPCTEMVADVNL PAAQLQIAMGIPLYRIKDIRMMYGVSPWGDSPIDFEDSA -
ACC2BC	LNPRLQVEHPCTEMIADVNL PAAQLQIAMGVPLHRLKDIRLLYGESPWG-----VTP
	***** * * * * * ***** * * * * *

FIG. 13 (part 4 of 5)

ustilagoBC	ESFKTQRKPQ-PQGHVVACRITAENPDTGFKPGMGALTELNFRSSTSTWGYFSVGTSGAL
ACC1BC	-----HVPC-PRGHVIAARITSENPDGFKPSSGTVQELNFRSNKNVWGYFSVAAAGGL
ACC2BC	ISFETPSNPPLARGHVIAARITSENPDGFKPSSGTVQELNFRSSKNVWGYFSVAATGGL
	* *** * * * * * * * * *
ustilagoBC	HEYADSQFGHIFAYGADRSEARKQMVISLKELSIRGDFRTTVEYLIKLLTDAFESNKIT
ACC1BC	HEFADSQFGHCFSWGENREEAISNMVVALKELSIRGDFRTTVEYLIKLLTESFQMNRID
ACC2BC	HEFADSQFGHCFSWGENRKEAISNMVVALKELSIRGDFRTTVEYLINLLETESFQNNID
	** ***** * * * * * * * * *
ustilagoBC	TGWL DGLIQDRLTAERPPADLAV (SEQ ID NO: 2)
ACC1BC	TGWL DRLIAEKVQAERPDTMLGV (SEQ ID NO: 10)
ACC2BC	TGWL DYLIAEKVQ-EKPDIMLGV (SEQ ID NO: 12)
	***** ** * * *

FIG. 13 (part 5 of 5)

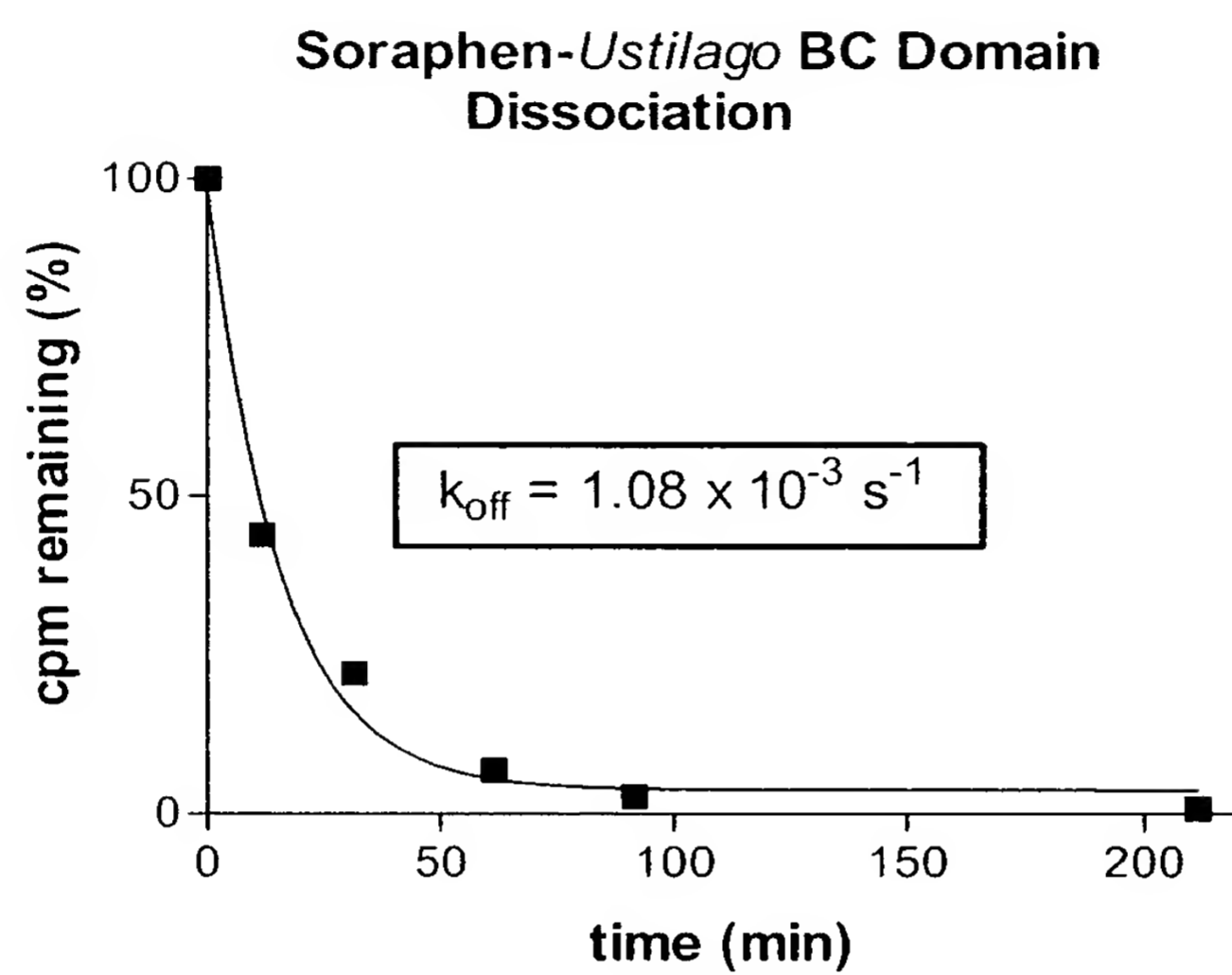
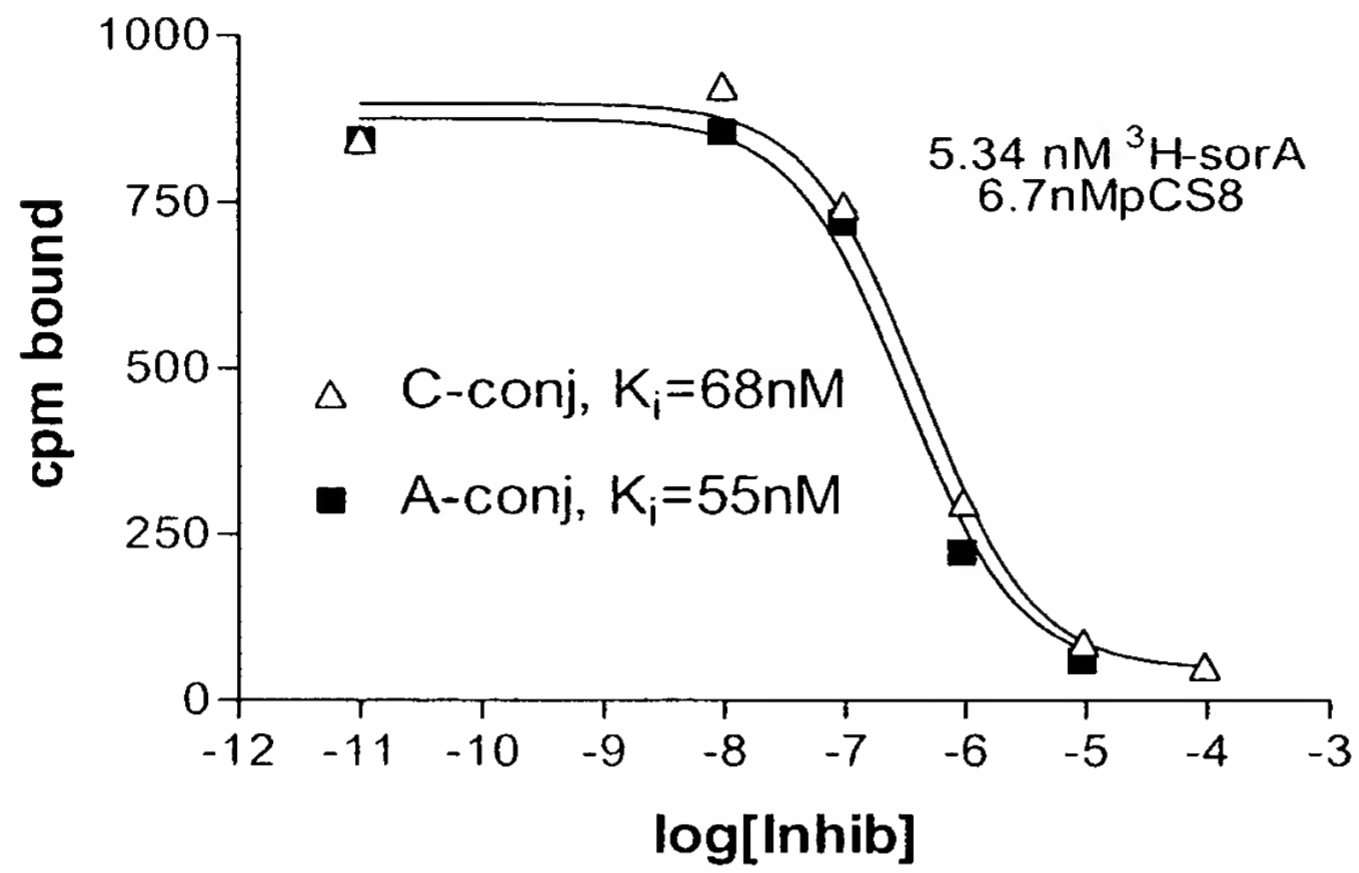


FIG. 14

A

Competition binding of soraphen-conjugates
to *Ustilago* BC domain



B

Competition binding of soraphen-conjugates
to full-length *Ustilago* ACCase

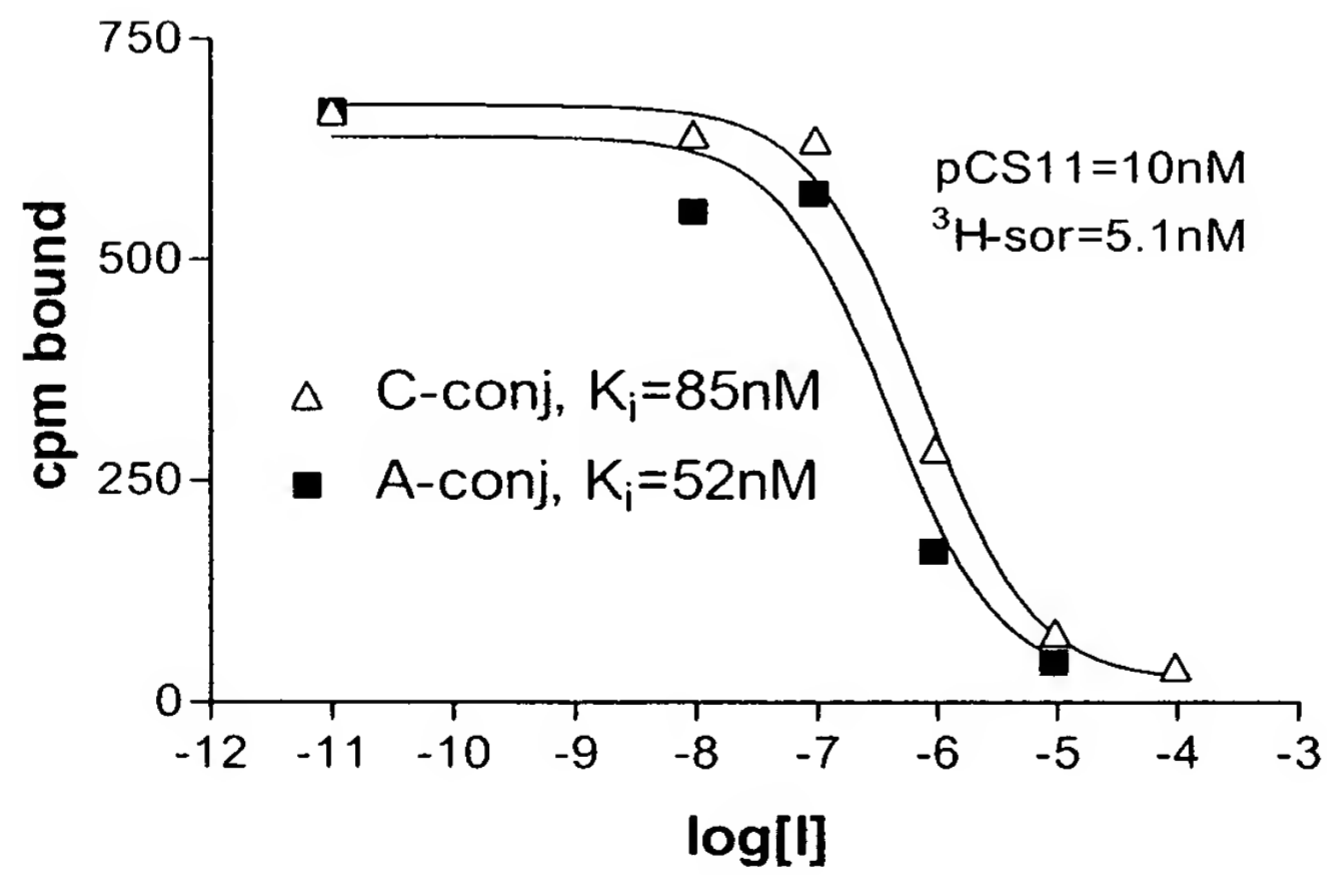


FIG. 15

Soraphen binding to wild-type and mutant *S. cerevisiae* BC domains

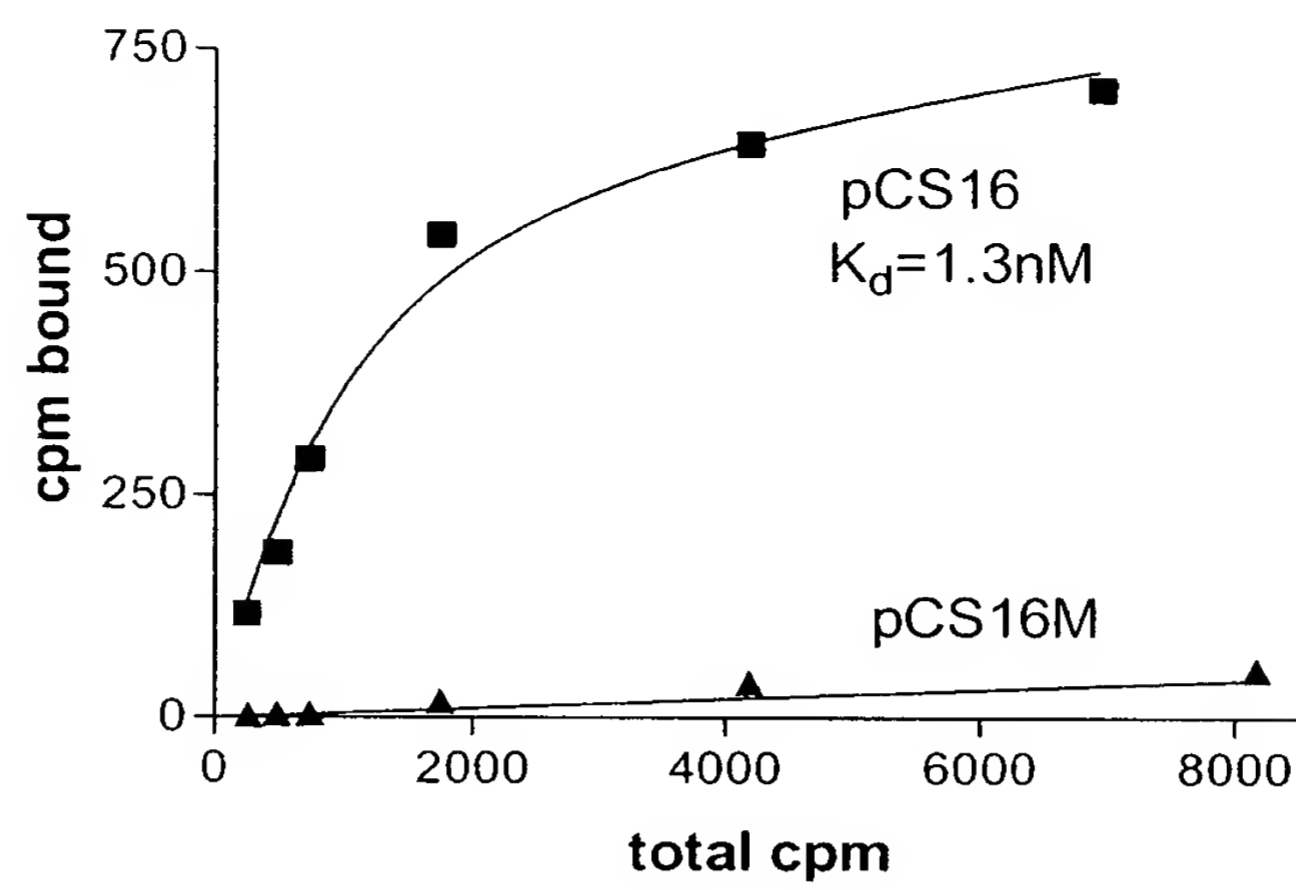


FIG. 16

Soraphen binding to wild-type and mutant *S. cerevisiae* ACCase

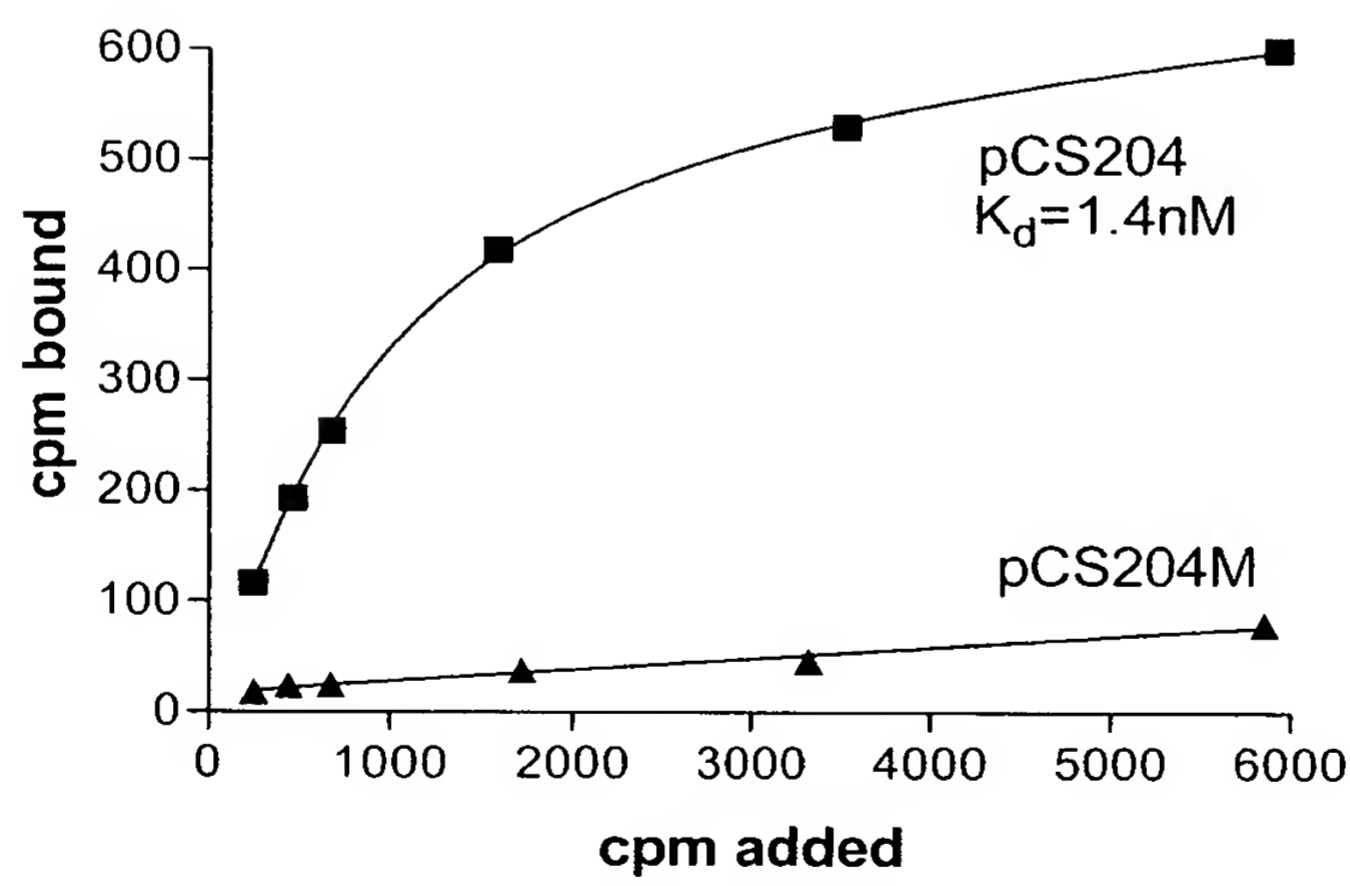


FIG. 17